SECTION Va—FOREST SOILS

The Rate of Respiration of Jack Pine Root Tips as Influenced by Extracts from Different Types of Humus[1]

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IN THIS study the influence of water extracts from different types of forest humus on the respiratory processes of jack pine root tips was observed, using manometric techniques. Since the uptake of oxygen and release of carbon dioxide reflect in part the metabolic activity of living cells (2), the investigation aimed to elucidate the effect of humus suspensions on the early development of seedlings and their probable behavior under conditions of natural reproduction.

Cold water extracts were prepared by treating 100 cc of humus (A_0 or A_1 horizon) with an equal amount of distilled water. The suspension was vigorously stirred and allowed to remain at room temperature for 24 hours. Then, the suspension was filtered through a coarse filter paper and sterilized by means of a Seitz filter. The sterilized filtrate was kept in the refrigerator until used.

The seeds of jack pine were surface sterilized with 1% formaldehyde solution and germinated in sterile Petri dishes on quartz sand. The active metabolic part of the root was cut off, placed in distilled water, and stored at about 40°F for 12 to 24 hours.

The standard procedure for determination of oxygen uptake was followed using the rotary Lardy–Warburg apparatus (3). About 50 mg of root tips were placed in a respiratory flask with 1 ml of phosphate buffer (0.05 M.). Because the activity of the enzyme system is influenced by the reaction of the medium (2), the reaction of the buffer solution was adjusted to that of the humus extract. The liberated CO_2 was absorbed by 0.2 ml of 20% KOH in the well of the respiratory flask. One milliliter of the humus extract was placed in the side arm of the flask. After 1 hour the system attained equilibrium and was brought in contact with the root tissue. The temperature of the water bath of the respirometer was maintained at 30°C. The oxygen uptake was calculated in microliters taken up per mg of dry weight of tissue per hour (Qo_2). The readings were taken every 15 minutes and each experiment was continued for at least 2½ hours.

In spite of all precautions, in trials with root tips *in toto* considerable variations were observed between duplicates. These were traced to differences in the degree of lignification of cell membranes affecting the metabolic activity. In consequence, 1 gram of root tissue was homogenized in 75 ml of distilled water for 5 minutes using a Waring blendor. In all cases, the activity of the homogenate by far exceeded that of the root tips due to an increased surface of ruptured tissues and greater accessibility of the humus extract to liberated enzyme systems.

In a preliminary investigation, the effect of extracts of two types of humus on homogenized root tips of jack pine were studied in duplicate, repeating experiments three times with homogenates prepared at weekly intervals. The extracts were obtained from jack pine matted mor and black spruce matted mor. The results, presented in Fig. 1, are expressed in terms of relative stimulation, i.e., the ratio of oxygen uptake in humus suspensions to oxygen uptake in buffer solution. These experiments give a measure of the variability of analytical data ob-

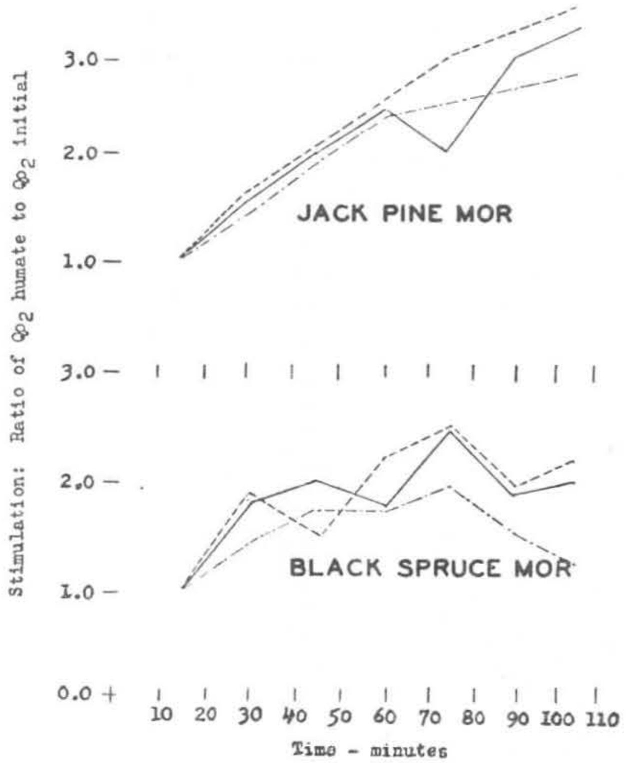

FIG. 1.—Variation in the relative stimulation of homogenized jack pine root tips under the influence of water extracts of humus from jack pine matted mor and black spruce matted mor. The individual lines present results obtained with homogenates prepared at weekly intervals.

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tained with the same type of humus and homogenized root tips of the same seed origin.

In further course of study, extracts were prepared from four humus types of radically different composition: jack pine sand mull (Plainfield series), jack pine matted mor (podzolic phase, Hiawatha series), black spruce matted mor (sandy outwash podzol with *Hypnum* cover), and oak crumb mull (Miami series). The samples of the latter two types were collected under stands of a very low rate of growth. The cumulative effect of each of these extracts on jack pine root homogenate is presented in Fig. 2. Considering the micrometric nature of the apparatus, the results show a pronounced difference between the effect of humus types originated under jack pine and other species. It should be noted that in

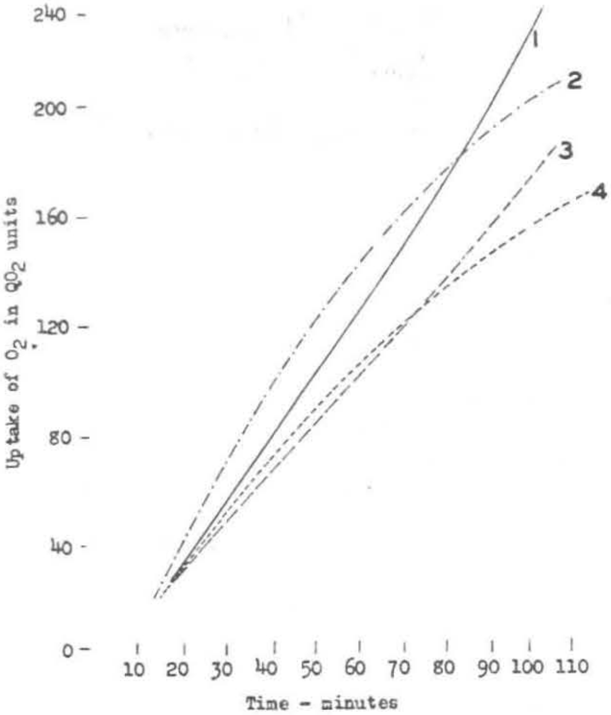

FIG. 2.—Cumulative uptake of O_2 by homogenized jack pine root tips under the influence of water humus extracts from the following humus types: 1—black spruce matted mor; 2—jack pine matted mor; 3—jack pine sand mull; 4—oak crumb mull. Results are calculated in microliters per hour and milliliter of homogenate.

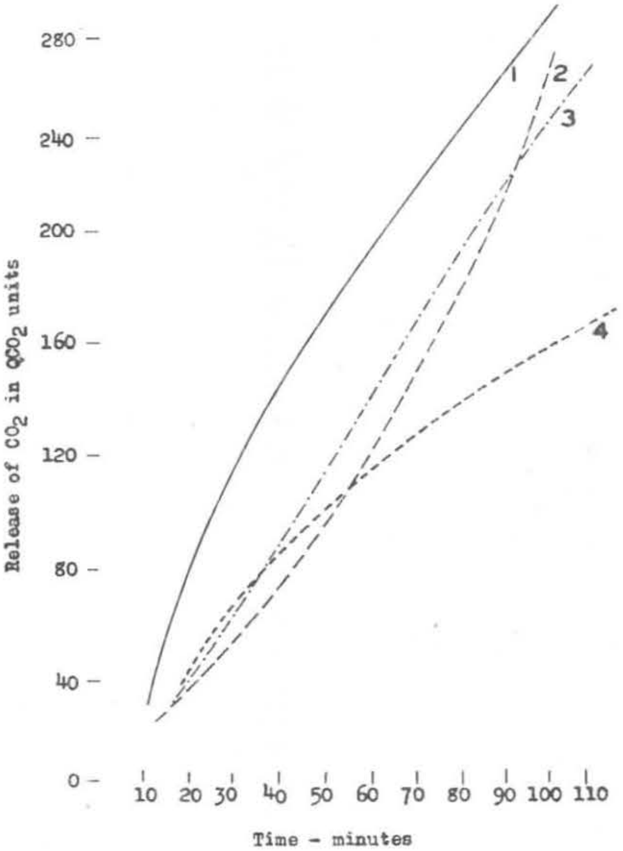

FIG. 3.—Cumulative release of CO_2 by homogenized jack pine root tips under the influence of water humus extracts from the following humus types. 1—black spruce matted mor; 2—jack pine matted mor; 3—jack pine sand mull; 4—oak crumb mull. Results are calculated in microliters per hour and milliliter of homogenate.

TABLE 1.—*Uptake of oxygen and release of carbon dioxide by homogenized root tips of jack pine under the influence of water extracts from mull and mor types of humus originated on stands of widely different productivity.*

Type of humus and location	Reaction of buffer pH	Initial QO_2	Maximum QO_2	Maximum stimulation in O_2 uptake	Initial QCO_2	Maximum QCO_2	Maximum stimulation in CO_2 release
Jack pine sand mull; Big Flats, Wis.	5.0	16.7	40.0	2.40	26.3	44.8	1.70
Jack pine matter mor; Buffalo Lake, Wis.	4.0	17.5	50.0	2.86	27.2	53.3	1.96
Maple-yellow birch coarse mull; Duschesnay, Quebec	5.0	5.0	37.5	7.50	12.2	36.2	3.00
Balsam fir friable mor; Laurentides Park, Quebec	3.5	3.1	20.1	6.45	13.1	27.8	2.12
Black spruce matted mor; Laurentides Park, Quebec	3.5	15.7	38.1	2.43	21.9	93.4	4.26
Oak crumb mull; Monroe, Wis.	7.0	15.0	32.4	2.16	14.2	55.9	3.94

TABLE 2.—*Respiratory quotient of homogenized root tips of jack pine under the influence of water extracts from mull and mor types of humus originated in stands of widely different productivity.*

Type of humus and location	Time, minutes						
	15	30	45	60	75	90	105
	Respiratory quotient (QCO_2:QO_2) at different intervals						
Jack pine sand mull; Big Flats, Wis.	1.58	0.94	1.65	1.09	1.11	1.64	1.00
Jack pine matted mor; Buffalo Lake, Wis.	1.55	0.93	0.95	1.03	1.19	1.00	0.99
Maple–yellow birch coarse mull; Duschesnay, Quebec	2.41	1.61	1.51	1.06	0.92	0.61	0.89
Balsam fir friable mor; Laurentides Park, Quebec	2.50	1.75	2.00	1.50	1.49	1.06	0.94
Black spruce matted mor; Laurentides Park, Quebec	1.40	3.20	1.05	1.22	0.93	1.02	0.88
Oak crumb mull; Monroe, Wis.	0.95	0.89	2.48	0.80	0.15	1.25	0.40

all trials the effect of humus suspensions was characterized by two peaks in the uptake of oxygen, apparently caused by two stimulatory factors.

The investigations of oxygen uptake were supplemented by the study of carbon dioxide evolution. The release of CO_2 by homogenized root tips under the influence of humus suspensions was determined by the direct method (3). Since the reaction of buffers higher than pH 5.0 leads to the formation of bicarbonate and hence low manometric readings, the flask constants were recalculated to the effective values for the CO_2 solubility.

The extracts of the same four humus types were used in determination of CO_2 evolution. The results are presented in Fig. 3. The shape of the curves indicates that humus types originated under jack pine induce a normal evolution of carbon dioxide. On the other hand, both oak crumb mull and black spruce mor bring about an accelerated release of carbon dioxide followed by significantly retarded activity of the enzymatic system.

In order to establish the increase in the rate of metabolic activity due to addition of humus suspensions, the values for the maximum O_2 uptake were related to the values of O_2 uptake before the addition of humus. The ratio thus established expresses the degree of stimulation produced by humus extract. A similar procedure was followed to determine the stimulation in regard to release of carbon dioxide.

In comparison of the degree of stimulation, the previous four types of humus were supplemented by hard maple-yellow birch coarse mull (alluvial deposit) and balsam fir friable mor (sandy outwash podzol with *Hylocomium* cover). Hence, two humus types originated under jack pine (sandy mull and matted mor) were compared with two types formed under highly productive stands (hard maple-yellow birch coarse mull and balsam fir friable mor) and two types formed under stands of extremely low rate of growth (oak crumb mull and black spruce matted mor). The results are presented in Table 1.

As the results indicate, types originated under jack pine give about the same stimulation for O_2 uptake and CO_2 evolution. Other types induce widely different reactions. Spruce matted mor and oak crumb mull, i.e., types associated with poorly growing forest stands, effect a low stimulation in uptake of oxygen and a high stimulation in release of carbon dioxide. Balsam fir friable mor and hard maple-yellow birch coarse mull produce the opposite effects.

For the purpose of obtaining an over-all picture of the prevailing trends in metabolism of the homogenate, the respiratory quotients were calculated for the same six types of humus. These values were obtained as the ratio between CO_2 evolution and O_2 uptake. The results, presented in Table 2, indicate that the humus types originated under jack pine and humus types from productive forest stands induce a normal course of metabolism in homogenized tissues. The types originated under poorly growing stands, however, show higher respiratory quotients immediately after addition of humus extract, i.e., a sign of pronounced decarboxylation. The breakdown of carboxyl groups of the humus suspension by the homogenate's decarboxylases and subsequent depletion of the enzymatic system indicate a general inhibition rather than stimulation of growth.

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